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16. ABSTRACT

Chloroform administered intraperitoneally (i.p.) to male mice and rats resulted in a dose-dependent increase in hepatic ornithine decarboxylase (ODC) activity. Maximal induction of the enzyme in mice was 10-fold and occurred at 375 mg/kg chloroform; in rats it was 52-fold and occurred at 750 mg/kg chloroform. Chloroform increased in mice and decreased in rats the rate of hepatic and renal DNA synthesis. Therefore, the induction of ODC activity in rat liver was not followed with an increase in DNA synthesis. The implications of these results to the proposed nongenetic mechanism of chloroform induction of hepatocellular carcinoma in mice and renal tumors in rats are discussed.

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EFFECT OF CHLOROFORM ON HEPATIC AND RENAL DNA SYNTHESIS AND ORNITHINE DECARBOXYLASE ACTIVITY IN MICE AND RATS

(Cell proliferation; tumor enhancement; hepatotoxicity)

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SUMMARY

Chloroform administered intraperitoneally (i.p.) to male mice and rats resulted in a dose-dependent increase in hepatic ornithine decarboxylase (ODC) activity. Maximal induction of the enzyme in mice was 10-fold and occurred at 375 mg/kg chloroform; in rats it was 52-fold and occurred at 750 mg/kg chloroform. Chloroform increased in mice and decreased in rats the rate of hepatic and renal DNA synthesis. Therefore, the induction of ODC activity in rat liver was not followed with an increase in DNA synthesis. The implications of these results to the proposed nongenetic mechanism of chloroform induction of hepatocellular carcinoma in mice and renal tumors in rats are discussed.

INTRODUCTION

ODC (EC 4.1.1.17) is the rate-limiting enzyme in polyamine biosynthesis [1]. The endogenous level of ODC activity in rat liver is very low and the half-life of ODC is 10–15 min which makes it one of the shortest lived enzyme [2–4]. ODC undergoes very rapid induction in cells and tissues that have been stimulated to divide [2–4]. The increase in ODC activity is followed by an increase in polyamine biosynthesis which in turn is followed by an increased in DNA, RNA and protein biosynthesis.

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Abbreviations: ODC, ornithine decarboxylase; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate.

Since the amount of ODC activity in a cell can be very rapidly modulated and is apparently associated with macromolecular biosynthesis, it has been proposed that the level of ODC in a cell might serve an important function in the regulation of cell proliferation and macromolecular biosynthesis [3–4].

Chloroform is toxic to the liver and kidney [5,6] and is a carcinogen to the liver of mice and the kidney of rats [7]. The two distinct stages of initiation and promotion have been described for the process of chemical carcinogenesis [8–12]. Initiation is believed to require a genetic event, whereas promotion is a non-genetic phenomenon [2]. The carcinogenic mechanism of chloroform has been proposed to be non-genetic and to be associated with a regenerative hyperplasia in response to hepatic and renal toxicity [13,14]. Thereby, chloroform would act on initiated cells to enhance the incidence of cancer. In this communication, we report the dose–response relationship of the effect of chloroform on hepatic and renal DNA synthesis and ODC activity in mice and rats as an estimate of the relationship for chloroform induced hyperplasia.

MATERIAL AND METHODS

Glass distilled chloroform (without preservative) and carbon tetrachloride were purchased from Burdick and Jackson Laboratories (Muskegon, MI) and [6-^{3}H]thymidine (70 Ci/mmol) and DL-[1-^{14}C]ornithine hydrochloride (51.3 mCi/mmol) from New England Nuclear (Boston, MA). Male Fisher-344 albino rats 175–225 g and male B6C3F1 mice (7–9 weeks) purchased from Charles River Company (Portage, MI) were used in these studies. They were fed Purina Laboratory Chow (Ralston Purina Co., St. Louis, MO) and given distilled water ad lib. The animals were maintained in accordance with the guidlines set forth in 'The Maintenance and Care of Laboratory Animals' by the National Research Council, National Academy of Science, USA.

For determination of ODC activity, the animals were killed by decapitation between 9:00 and 10:00 a.m. and 18 h after they received an i.p. dose of chloroform. We have previously determined that the peak in chloroform induction of ODC occurred at 18 h [15]. ODC activity was determined as previously described [16] by the procedure of Bethel and Pegg [17], which measured the amount of $^{14}CO_2$ liberated from DL-[1-^{14}C]ornithine. Protein was determined by the Bradford procedure [18] employing a BioRad protein assay kit (BioRad, Richmond, CA). ODC activity was expressed as pmol $^{14}CO_2$ liberated/mg protein/30 min.

DNA synthesis was determined in animals that received i.p. chloroform 24 h and 200 Ci/kg [^{3}H]thymidine 2 h prior to termination. Approx 100 mg of liver or kidney was homogenized in ice-cold 2.5 ml of 0.2 N perchloric acid. After centrifugation, the precipitate was resuspended and washed three times in ice-cold 0.5 N perchloric acid and once in ice-cold ethanol containing 2% sodium acetate. The DNA was extracted twice with 0.5 N perchloric acid, at 90°C for 15 min. An aliquot of the ex-

tract was used to determine the DNA concentration by the diphenylamine procedure of Burton [19] and a second aliquot (0.5 ml) after the addition of 15 ml ACS containing 0.1 ml of 1.9 M potassium bicarbonate was used to measure [^{3}H]thymidine in a Beckman model 9000 liquid scintillation counter (Beckman Instrument Co., Fullerton, CA).

At killing, blocks of liver and kidney were obtained and fixed in buffered formalin, hematoxylin and eosin slides of liver and kidney were prepared and examined for histopathology.

RESULTS

The dose–response relationship of chloroform induction of hepatic ODC activity in mice and rats is presented in Fig. 1. Chloroform caused a dose-related increase of ODC activity in the liver of both mice and rats. The response in rats was greater than in mice. In rats, 750 mg/kg chloroform yielded a maximal 52-fold stimulation of ODC activity. In mice, maximal stimulation of a 10-fold increase in ODC activity occurred at 375 mg/kg chloroform. Higher doses of chloroform resulted in a less than maximal stimulation in ODC activity. In both species, a small but significant stimulation of ODC activity occurred between 100 and 200 mg/kg chloroform.

The effect of chloroform on hepatic and renal DNA synthesis was determined (Fig. 2). In rats, the rate of hepatic and renal DNA synthesis decreased with increasing dose of chloroform administered 24 h prior to termination. In another experiment, the rate of hepatic and renal DNA synthesis was also slightly decreased when measured 24, 36 or 48 h after the administration of chloroform. In mice, 50 mg/kg chloroform when administered 24 h prior to termination yielded a maximal stimulation of DNA synthesis of 3.1- and 6.7-fold in liver and kidney, respectively. In both organs larger doses of chloroform resulted in a less than maximal increase in DNA synthesis and doses of chloroform 375 mg/kg appeared to result in a rate of DNA synthesis that was below control level.

Minimal microscopic changes were detected in the liver and kidney. In rat liver, treatment related alterations included (1) a minimal to moderate increase in basophilia of the cytoplasm, and (2) a scattering among the groups of a few animals with peritonitis and foci of inflammation. There was no evidence of treatment related toxicity in rat kidney. In the mouse liver, a few of the animals that received 200, 400 or 750 mg/kg chloroform exhibited minimal to moderate cytoplasmic swelling and basophilia and focal inflammation. There was treatment related renal changes in mice that included tubular nephrosis and dilatation and hyaline casts within tubules.

DISCUSSION

The effect of chloroform on ODC activity and DNA synthesis was investigated in rat and mouse liver and kidney. In mice and rats, dose-dependent increase in

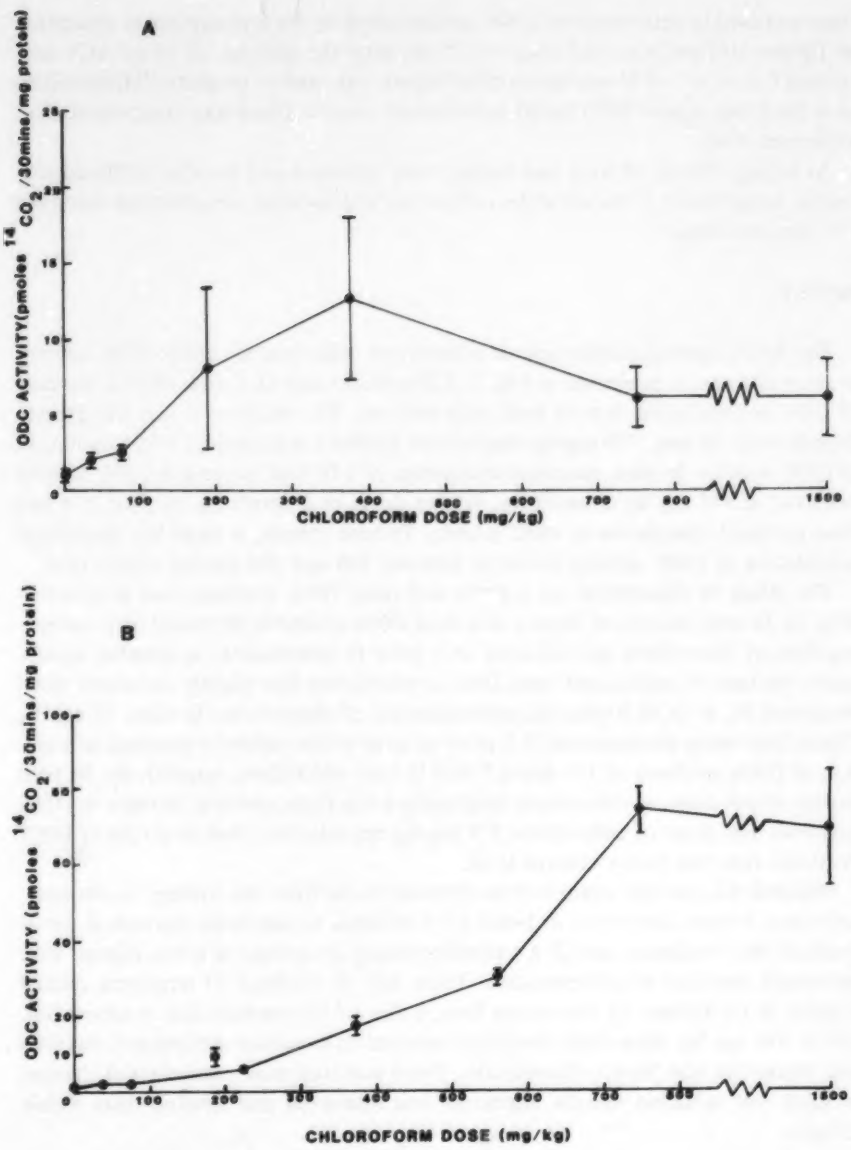

Fig. 1. Dose–response relationship of chloroform induction of hepatic ODC activity. Rats received i.p. the appropriate dose of chloroform and were killed 18 h later. The points represent the mean ± SE of a minimum of 8 animals except for the 1500 mg/kg chloroform in rats where only 2 of 8 animals survived until termination. (A) mice; (B) rats.

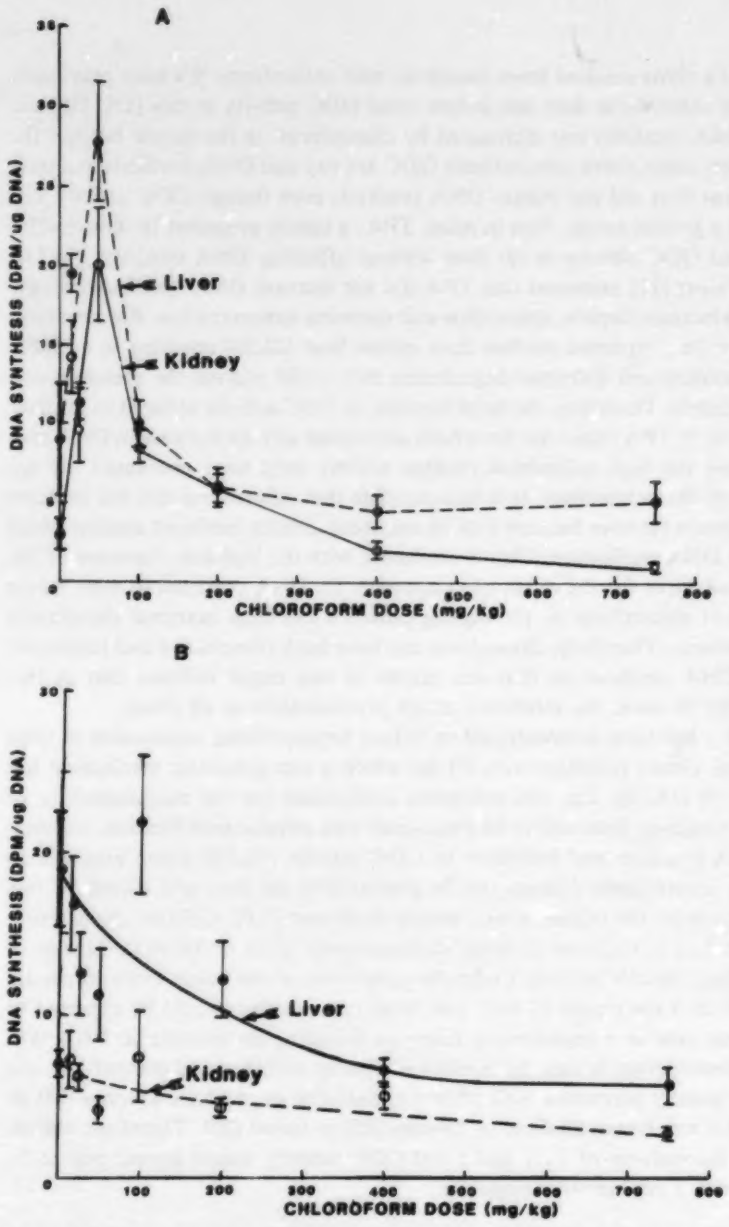

Fig. 2. Dose–response relationship of chloroform induction of hepatic and renal DNA synthesis. The animals received i.p. the appropriate dose of chloroform and were killed 24 h later. 2 h prior to termination the animals also received i.p. 200 Ci/kg [^{3}H]thymidine. The points represent the means of 5 to 8 animals ± SE. (A) mice; (B) rats.

hepatic ODC activity resulted from treatment with chloroform. We have previously reported that chloroform does not induce renal ODC activity in rats [15]. Hepatic and renal DNA synthesis was stimulated by chloroform, in the mouse but not the rat. Therefore, chloroform induced both ODC activity and DNA synthesis in mouse liver but in rat liver did not induce DNA synthesis even though ODC activity was increased to a greater extent than in mice. TPA, a tumor promoter in mouse skin, also increased ODC activity in rat liver without affecting DNA synthesis [20,21]. Byus and Weiner [21] proposed that TPA did not increase DNA synthesis because TPA did not increase hepatic spermidine and spermine concentration. Rat liver contains much more polyamine oxidase than mouse liver [22,23] resulting in a higher rate of spermidine and spermine degradation that could prevent the accumulation of the polyamines. Therefore, the large increase in ODC activity induced in rat liver by chloroform or TPA might not have been associated with an increase in DNA synthesis because the high polyamine oxidase activity may have prevented the accumulation of the polyamines. It is also possible that chloroform did not increase DNA synthesis in rat liver because it or its resultant toxicity inhibited another event required for DNA replication. This is consistent with the biphasic character of the dose–response curve for the effect of chloroform on DNA synthesis in mice where a dose level of chloroform $>$ 100 mg/kg caused a less than maximal stimulation of DNA synthesis. Therefore, chloroform can have both stimulatory and inhibitory effects on DNA synthesis so that our results in rats might indicate that in this species, unlike in mice, the inhibitory effect predominates at all doses.

Chloroform has been demonstrated to induce hepatocellular carcinomas in mice and epithelial kidney tumors in rats [7] for which a non-genotoxic mechanism has been proposed [13,14]. The non-genotoxic mechanism for the carcinogenicity of chloroform has been proposed to be associated with cellular proliferation, stimulation of DNA synthesis and induction of ODC activity [13,14]. Upon exposure to chloroform, severe tissue damage can be produced in the liver and kidney of rats and mice which are the organs where tumors developed [5,6]. Cellular regeneration and proliferation in response to tissue damage could result in the enhancement of tumor incidence by chloroform. Under the conditions of our study, chloroform did not cause at 24 h the extent of liver and renal necrosis that would be expected to result by that time in a regenerative response including an increase in DNA synthesis. To demonstrate in rats the toxicity to acutely administered chloroform, the animals are usually pretreated with phenobarbital [24] or aliphatic ketones [25] or chemicals that can be metabolized to ketones [25] or fasted [26]. Therefore, the induction by chloroform of liver and renal ODC activity would appear not to be associated with a regenerative response.

REFERENCES

1 D.R. Morris and R.H. Fillingame, Regulation of amino acid decarboxylation, Annu. Rev. Biochem., 43 (1974) 303–325.

2 D.H. Russell and S.H. Synder, Amine synthesis in regenerating rat liver: extremely rapid turnover of ornithine decarboxylase, Mol. Pharmacol., 5 (1969) 253–262.

3 D.H. Russell and G.G.M. Durie, in Polyamines as Biochemical Markers of Normal and Malignant Growth, Raven Press, New York, 1978, pp. 1–178.

4 E.S. Canellakis, D. Viceps-Madore, D.A. Kyriakidis, and J.S. Heller, The regulation and function of ornithine decarboxylase and of the poly-amines, Curr. Topics Cell. Reg., 15 (1969) 156–202.

5 C.D. Klassen and G.L. Plaa, Relative effects of various chlorinated hydrocarbons on liver and kidney function in mice, Toxicol. Appl. Pharmacol., 9 (1966) 139–153.

6 L.R. Pohl, Biochemical toxicology of chloroform, Rev. Biochem. Toxicol., 1 (1979) 79–107.

7 National Cancer Institute. Report on carcinogenesis bioassay of chloroform, Natl. Tech. Inform. Service, PB, 264018/AS, 1/60, 1976.

8 M.A. Pereira, Rat liver foci bioassay, J. Am. Coll. Toxicol., 1 (1982) 101–118.

9 H.C. Pitot and A.E. Sirica, The stages of initiation and promotion in hepatocarcinogenesis, Biochim. Biophys. Acta, 605 (1980) 191–215.

10 S.L. Herren and M.A. Pereira, Tumor promotion in rat liver, Environ. Health Perspect., 50 (1983) In press.

11 E. Farber, The sequential analysis of liver cancer induction, Biochem. Biophys. Acta, 605 (1980) 149–166.

12 R.K. Boutwell, The function and mechanism of promoters of carcinogenesis, CRC Crit. Rev. Toxicol., 2 (1974) 419–443.

13 M.A. Pereira, L.-H.C. Lin, J.M. Lippitt, and S.L. Herren, Trihalomethanes as initiators and promoters of carcinogenesis, Environ. Health Perspect., 46 (1982) 151–156.

14 R.H. Reitz, T.R. Fox and J.F. Quast, Mechanistic considerations for carcinogenic risk estimation chloroform, Environ. Health Perspect., 46 (1982) 163–168.

15 R.E. Savage Jr., C. Westrich, C.W. Guion and M.A. Pereira, Chloroform induction of ornithine decarboxylase activity in rats, Environ. Health Perspect., 46 (1982) 157–162.

16 M.A. Pereira, R.E. Savage Jr., S.L. Herren and C.W. Guion, Comparison of enhancement of GGTase-positive foci and induction of ornithine decarboxylase in rat liver by barbiturates, Carcinogenesis, 3 (1982) 147–150.

17 D.R. Bethell and A.E. Pegg, Effects of diamines on the activity of ornithine decarboxylase in control and virally transformed 3T3 fibroblasts, Biochem. J., 180 (1979) 87–94.

18 M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding, Anal. Biochem., 72 (1976) 248–254.

19 K. Burton, A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid, Biochem. J., 62 (1956) 315–323.

20 R.A. Weiner and C.V. Byrus, Induction of ornithine decarboxylase by 12-*O*-tetradecanoylphorbol-13-acetate in rat tissue, Biochem. Biophys. Res. Commun., 97 (1980) 1575–1581.

21 C.V. Byus and R.A. Weiner, Tumor promoting phorbol-ester derivatives increase ornithine decarboxylase activity and polyamine biosynthesis in the liver of the rat and mice, Carcinogenesis, 3 (1982) 751–755.

22 F.N. Bolkenius and N. Seiler, Acetyl derivaties as intermediates in polyamine catabolism, Int. J. Biochem., 13 (1981) 287–292.

23 A. Poso and A.E. Pegg, Effect of carbon tetrachloride on polyamine metabolism in rodent liver, Arch. Biochem. Biophys., 217 (1982) 730–737.

24 L.R. Pohl and G. Krishra. Deterium isotope effect in bioactivation and hepatotoxicity of chloroform, Life Sci., 23 (1978) 1067–1072.

25 W.R. Hewitt, H. Miyajima, M.G. Coté and G.L. Plaa, Acute alteration of chloroform-induced hepato and nephrotoxicity by *n*-hexane, methyl-*n*-butyl ketone and 2,5-haxanedione, Toxicol. Appl. Pharmacol., 53 (1980) 30–248.

26 D.N. McMartin, J.A. O'Connor Jr. and L.S. Kaminsky, Effects of differential changes in rat hepatic and renal cytochrome P-450 concentrations on hepatotoxicity, and nephrotoxicity of chloroform, Res. Commun. Chem. Pathol. Pharmacol., 31 (1981) 99–110.